

(FILE 'HOME' ENTERED AT 10:35:54 ON 16 FEB 2000)

FILE 'CAPLUS, MEDLINE' ENTERED AT 10:36:13 ON 16 FEB 2000
L1 517 S (THERMOTOGA OR THERMATOGA)(2W)(NEAPOLITANA OR MARITIMA)
L2 25 S L1(5A)(DNA POLYMERASE#)
L3 22 DUPLICATE REMOVE L2 (3 DUPLICATES REMOVED)

=> d 1-22 ibib ab

L3 ANSWER 1 OF 22 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:46954 CAPLUS
TITLE: Thermostable DNA polymerases from Thermotoga and
mutants and their use in DNA sequencing and
amplification
INVENTOR(S): Hughes, A. John; Chatterjee, Deb K.
PATENT ASSIGNEE(S): Life Technologies, Inc., USA
SOURCE: U.S., 65 pp., Cont.-in-part of U. S. Ser. No. 689,818,
abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------------------|------|----------|-----------------|----------|
| US 6015668 | A | 20000118 | US 1996-706706 | 19960906 |
| US 5912155 | A | 19990615 | US 1995-370190 | 19950109 |
| US 5939301 | A | 19990817 | US 1995-537400 | 19951002 |
| PRIORITY APPLN. INFO.: | | | US 1994-316423 | 19940930 |
| | | | US 1995-370190 | 19950109 |
| | | | US 1995-525057 | 19950908 |
| | | | US 1995-537397 | 19951002 |
| | | | US 1995-537400 | 19951002 |
| | | | US 1995-576759 | 19951221 |
| | | | US 1996-689818 | 19960814 |

AB The method of synthesizing, sequencing, and amplifying a double strand DNA using the Thermotoga DNA polymerase and the kit required are disclosed. The invention relates to a thermostable ***DNA*** ***polymerase*** from ***Thermotoga*** ***neapolitana*** (Tne) and mutants. The mutant DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'.fwdarw.5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'.fwdarw.3' exonuclease activity of said DNA polymerase; (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides. The present invention also relates to the cloning and expression of the wild type or mutant DNA polymerases in E. coli, to DNA mols. contg. the cloned gene, and to host cells which express said genes.

L3 ANSWER 2 OF 22 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:549391 CAPLUS
DOCUMENT NUMBER: 131:167095
TITLE: Mismatch cleavage enzymes from extreme thermophiles

and their uses in molecular biology techniques
INVENTOR(S): Chirikjian, Jack G.; Bazar, Leonard S.; George, Albert
L.
PATENT ASSIGNEE(S): Trevigen, Inc., USA
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9942595 | A1 | 19990826 | WO 1999-US3274 | 19990219 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 9927664 | A1 | 19990906 | AU 1999-27664 | 19990219 |
| PRIORITY APPLN. INFO.: US 1998-PV75194 19980219 | | | | |
| US 1998-75194 19980219 | | | | |
| WO 1999-US3274 19990219 | | | | |

AB The present invention is directed to extreme thermophilic mismatch cleavage enzymes and their uses. The gene sequence encoding Thermotoga maritima endonuclease V (TM-EndoV, also known as deoxyinosine 3'-endonuclease) is provided. TM-EndoV exhibits extreme thermophilic mismatch cleavage activity, does not exhibit resolvase activity, does not require a GATC sequence to effectuate mismatch cleavage, and does not require a divalent cation to effectuate cleavage. In one embodiment, TM-EndoV cleaves A/G, C/C, G/G, T/C, A/C, A/A, and T/T mismatches, but does not cleave T/G mismatches or a bubble formation caused by an insertion or deletion mutation. When used in conjunction with an enzyme specificity altering agent such as DMSO, however, TM-EndoV does cleave at a bubble formation caused by an insertion or deletion mutation and cleaves T/G mismatches. TM-EndoV may be used for cleavage of mismatches in the detection of mutations by probe hybridization, detecting a sequence in a target polynucleotide, and cleaving mismatches created during PCR.

L3 ANSWER 3 OF 22 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:166632 CAPLUS
DOCUMENT NUMBER: 130:205910
TITLE: High fidelity polymerases and uses thereof
INVENTOR(S): Yang, Shuwei; Chatterjee, Deb K.
PATENT ASSIGNEE(S): Life Technologies, Inc., USA
SOURCE: PCT Int. Appl., 84 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9910366 | A1 | 19990304 | WO 1998-US17810 | 19980828 |
| W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| AU 9891235 | A1 | 19990316 | AU 1998-91235 | 19980828 |
| PRIORITY APPLN. INFO.: US 1997-56263 19970829 | | | | |
| US 1997-60131 19970926 | | | | |
| US 1998-85247 19980513 | | | | |
| US 1998-141522 19980827 | | | | |
| WO 1998-US17810 19980828 | | | | |

AB The present invention relates to a DNA and RNA polymerases which have increased fidelity (or reduced misincorporation rate). In particular, the invention relates to a method of making such polymerases by modifying or mutating in the nucleotide binding domain (Arg722 and/or Lys726 in ***Thermotoga*** ***neapolitana*** ***DNA*** ***polymerase***) of the polymerase (e.g., the O-helix). Such modifications include those which (1) substantially reduce 3'.fwdarw.5' exonuclease activity; (2) enhance or increase the ability of the polymerase to incorporate dideoxynucleotides into a DNA mol. about as efficiently as deoxynucleotides; and (3) substantially reduce 5'.fwdarw.3' exonuclease activity. The invention also relates to DNA mols. contg. the genes encoding the polymerases of the invention, to host cells contg. such DNA mols. and to methods to make the polymerases using the host cells. The polymerases are particularly suited for nucleic acid synthesis, sequencing, amplification and cDNA synthesis.

L3 ANSWER 4 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:571763 CAPLUS

DOCUMENT NUMBER: 131:224443

TITLE: Cloned wild-type and mutant ***DNA***
 polymerases from ***Thermotoga***
 maritima and their use for DNA sequencing,
 amplification and other genetic methods

INVENTOR(S): Chatterjee, Deb K.

PATENT ASSIGNEE(S): Life Technologies, Inc., USA

SOURCE: U.S., 67 pp., Cont.-in-part of U.S. Ser. No. 689,807,
 abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 6

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|--|------|----------|-----------------|----------|
| US 5948614 | A | 19990907 | US 1996-706702 | 19960906 |
| US 5939301 | A | 19990817 | US 1995-537400 | 19951002 |
| PRIORITY APPLN. INFO.: US 1995-525057 19950908 | | | | |
| US 1995-537397 19951002 | | | | |

US 1995-537400 19951002
US 1995-576759 19951221
US 1996-689807 19960814
US 1994-316423 19940930
US 1995-370190 19950109

AB The invention relates to a substantially pure thermostable ***DNA***
polymerase from ***Thermotoga*** ***maritima*** (Tma) and
Thermotoga ***neapolitana*** (Tne) and mutants thereof. The
Tne DNA polymerase has a mol. wt. of about 100 kilodaltons and is more
thermostable than Taq DNA polymerase. The mutant DNA polymerase has at
least one mutation selected from the group consisting of (1) a first
mutation that substantially reduces or eliminates 3'.fwdarw.5' exonuclease
activity of the DNA polymerase; (2) a second mutation that substantially
reduces or eliminates 5'.fwdarw.3' exonuclease activity of the DNA
polymerase; (3) a third mutation in the O helix of the DNA polymerase
resulting in the DNA polymerase becoming non-discriminating against
dideoxynucleotides. The present invention also relates to the cloning and
expression of the wild type or mutant DNA polymerases in E. coli, to DNA
mols. contg. the cloned gene, and to host cells which express said genes.
The DNA polymerases of the invention may be used in well-known DNA
sequencing and amplification reactions.

L3 ANSWER 5 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:518259 CAPLUS

DOCUMENT NUMBER: 131:167101

TITLE: Cloned wild-type and mutant ***DNA***
polymerases from ***Thermotoga***
neapolitana and their use

INVENTOR(S): Hughes, A. John, Jr.; Chatterjee, Deb K.

PATENT ASSIGNEE(S): Life Technologies, Inc., USA

SOURCE: U.S., 35 pp., Cont.-in-part of U.S. Ser. No. 370,190.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 6

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| US 5939301 | A | 19990817 | US 1995-537400 | 19951002 |
| US 5912155 | A | 19990615 | US 1995-370190 | 19950109 |
| WO 9709451 | A1 | 19970313 | WO 1996-US14189 | 19960906 |
| W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM | | | | |
| AU 9672362 | A1 | 19970327 | AU 1996-72362 | 19960906 |
| EP 871775 | A1 | 19981021 | EP 1996-933753 | 19960906 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| US 5948614 | A | 19990907 | US 1996-706702 | 19960906 |
| US 6015668 | A | 20000118 | US 1996-706706 | 19960906 |
| PRIORITY APPLN. INFO.: | | | US 1994-316423 | 19940930 |

US 1995-370190 19950109
US 1995-525057 19950908
US 1995-537397 19951002
US 1995-537400 19951002
US 1995-576759 19951221
US 1996-689807 19960814
US 1996-689818 19960814
WO 1996-US14189 19960906

AB The invention relates to a substantially pure thermostable ***DNA***
polymerase from ***Thermotoga*** ***neapolitana*** (Tne)
and mutants thereof. The Tne DNA polymerase has a mol. wt. of about 100
kilodaltons and is more thermostable than Taq DNA polymerase. The mutant
Tne DNA polymerase has at least one mutation selected from the group
consisting of (1) a first mutation that substantially reduces or
eliminates 3'.fwdarw.5' exonuclease activity of said DNA polymerase; (2) a
second mutation that substantially reduces or eliminates 5'.fwdarw.3'
exonuclease activity of said DNA polymerase; (3) a third mutation in the O
helix of said DNA polymerase resulting in said DNA polymerase becoming
non-discriminating against dideoxynucleotides. The present invention also
relates to the cloning and expression of the wild type or mutant Tne DNA
polymerase in E. coli, to DNA mols. contg. the cloned gene, and to host
cells which express said genes. The Tne DNA polymerase of the invention
may be used in well-known DNA sequencing and amplification reactions.

L3 ANSWER 6 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:383989 CAPLUS

DOCUMENT NUMBER: 131:29296

TITLE: Cloned ***DNA*** ***polymerase*** from
Thermotoga ***neapolitana***

INVENTOR(S): Chatterjee, Deb K.; Hughes, A. John, Jr.

PATENT ASSIGNEE(S): Life Technologies, Inc., USA

SOURCE: U.S., 17 pp., Cont.-in-part of U.S. Ser. No. 316,423,
abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 6

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| US 5912155 | A | 19990615 | US 1995-370190 | 19950109 |
| CA 2174944 | AA | 19960411 | CA 1995-2174944 | 19951002 |
| WO 9610640 | A1 | 19960411 | WO 1995-US12358 | 19951002 |
| W: CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| EP 725827 | A1 | 19960814 | EP 1995-935150 | 19951002 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 09506783 | T2 | 19970708 | JP 1995-511997 | 19951002 |
| US 5939301 | A | 19990817 | US 1995-537400 | 19951002 |
| US 6015668 | A | 20000118 | US 1996-706706 | 19960906 |
| PRIORITY APPLN. INFO.: US 1994-316423 19940930 | | | | |
| US 1995-370190 19950109 | | | | |
| US 1995-525057 19950908 | | | | |
| US 1995-537397 19951002 | | | | |
| US 1995-537400 19951002 | | | | |

WO 1995-US12358 19951002
US 1995-576759 19951221
US 1996-689818 19960814

AB The invention relates to a substantially pure thermostable ***DNA***
polymerase from ***Thermotoga*** ***neapolitana*** (Tne).
The Tne DNA polymerase has a mol. wt. of about 100 kDa and is more
thermostable than Taq DNA polymerase. The present invention also relates
to the cloning and expression of the Tne DNA polymerase in Escherichia
coli, to DNA mols. contg. the cloned gene, and to host cells which express
said genes. The Tne DNA polymerase of the invention may be used in
well-known DNA sequencing and amplification reactions.

L3 ANSWER 7 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1998:550530 CAPLUS

DOCUMENT NUMBER: 129:186150

TITLE: Thermostable Thermotoga DNA polymerases and use for
analyzing or typing polymorphic nucleic acids

INVENTOR(S): Chatterjee, Deb K.; Solus, Joseph; Yang, Shuwei

PATENT ASSIGNEE(S): Life Technologies, Inc., USA

SOURCE: PCT Int. Appl., 189 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|------|-----------------|------|
|------------|------|------|-----------------|------|

| | | | | |
|------------|----|----------|----------------|----------|
| WO 9835060 | A1 | 19980813 | WO 1998-US2791 | 19980209 |
|------------|----|----------|----------------|----------|

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE,
DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG,
KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI,
FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM,
GA, GN, ML, MR, NE, SN, TD, TG

| | | | | |
|------------|----|----------|---------------|----------|
| AU 9863251 | A1 | 19980826 | AU 1998-63251 | 19980209 |
|------------|----|----------|---------------|----------|

PRIORITY APPLN. INFO.: US 1997-37393 19970207

WO 1998-US2791 19980209

AB Claimed are thermostable DNA polymerases based on Thermotoga polymerases,
their sequences, and their use for DNA amplification in polymorphism
typing assays. The present invention provides methods for use in
identifying, analyzing and typing polymorphic DNA fragments, particularly
minisatellite, microsatellite or STR DNA fragments. In particular, the
invention provides methods using DNA polymerases, more particularly
thermostable DNA polymerases, and most particularly Thermotoga polymerases
or mutants or derivs. thereof, whereby minisatellite, microsatellite or
STR DNA mols. may be amplified and analyzed for polymorphisms. The
invention also relates to polymerases having reduced, substantially
reduced, or eliminated ability to add non-template 3' nucleotides to a
synthesized nucleic acid mol. In accordance with the invention, such
redn. or elimination may be accomplished by modifying or mutating the
desired polymerase.

L3 ANSWER 8 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1998:430045 CAPLUS
DOCUMENT NUMBER: 129:77566
TITLE: Nucleic acid amplification using a reversibly
inactivated thermostable enzyme
INVENTOR(S): Birch, David Edward; Laird, Walter Joseph; Zoccoli,
Michael Anthony
PATENT ASSIGNEE(S): Roche Molecular Systems, Inc., USA
SOURCE: U.S., 20 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|----------|-----------------|----------|
| US 5773258 | A | 19980630 | US 1996-680283 | 19960711 |

AB A thermostable enzyme is provided which is reversibly inactivated by chem. modification, characterized in that an incubation of the chem. modified thermostable enzyme in an aq. buffer at alk. pH at a temp. .ltorsim.25.degree. results in no significant increase in enzyme activity in .ltorsim.20 min, and wherein incubation of the chem. modified enzyme in an aq. buffer, formulated to about pH 8-9 at 25.degree., at a temp. .gtorsim.50.degree. results in a .gtoreq.2-fold increase in enzyme activity in .ltorsim.20 min. The chem. modified thermostable enzyme can be used for the amplification of nucleic acids, whereby the activity of the inactivated enzyme is recovered by an incubation of the reaction mixt. at an elevated temp. prior to, or as part of, the amplification reaction. The modification reagent is a dicarboxylic acid anhydride such as maleic anhydride, citraconic anhydride, cis-aconitic anhydride, 2,3-dimethylmaleic anhydride, 3,4,5,6-tetrahydrophthalic anhydride, or exo-cis-3,6-endoxo-DELTA.4-tetrahydrophthalic anhydride. For example, citraconylation of Taq DNA polymerase yields a modified enzyme with the desired properties for nucleic acid amplification in a PCR system.

L3 ANSWER 9 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1998:154804 CAPLUS
DOCUMENT NUMBER: 128:189207
TITLE: Modified thermostable DNA polymerase and its use in
DNA sequence determination
INVENTOR(S): Gelfand, David Harrow; Kalman, Lisa Vivian; Reichert,
Fred Lawrence
PATENT ASSIGNEE(S): F. Hoffmann-La Roche A.-G., Switz.
SOURCE: Eur. Pat. Appl., 29 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| EP 823479 | A2 | 19980211 | EP 1997-113182 | 19970731 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| CA 2210951 | AA | 19980206 | CA 1997-2210951 | 19970801 |

| | | | | |
|-------------|----|----------|----------------|----------|
| NO 9703595 | A | 19980209 | NO 1997-3595 | 19970805 |
| BR 9704260 | A | 19980915 | BR 1997-4260 | 19970805 |
| US 5939292 | A | 19990817 | US 1997-906484 | 19970805 |
| AU 9733197 | A1 | 19980212 | AU 1997-33197 | 19970806 |
| JP 10066588 | A2 | 19980310 | JP 1997-212350 | 19970806 |

PRIORITY APPLN. INFO.: US 1996-23376 19960806

OTHER SOURCE(S): MARPAT 128:189207

AB The invention provides thermostable DNA polymerase enzymes that comprises the amino acid sequence SerGlnIleXaaLeuArgXaa, wherein "Xaa" at position 4 of this sequence is any amino acid residue but not a glutamic acid residue (Glu), preferably a glycine residue and "Xaa" at position 7 of this sequence is a valine residue (Val) or an isoleucine residue (Ile). The thermostable DNA polymerases of the invention have enhanced efficiency for incorporating unconventional nucleotides, such as ribonucleotides, into DNA products and are advantageous in many in vitro synthesis applications. Such enzymes are particularly useful for use in nucleic acid sequencing protocols and provide novel means for DNA sequence anal. with cost and efficiency advantages. Also claimed are nucleic acids encoding said polymerases, vectors and host cells comprising such a nucleic acid, as well as compns. for use in a DNA sequencing reaction, kits and methods for sequencing including such polymerases. The DNA polymerase gene of *Thermus aquaticus* is revealed.

L3 ANSWER 10 OF 22 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 1

ACCESSION NUMBER: 1999:26518 CAPLUS

DOCUMENT NUMBER: 130:207163

TITLE: The hyperthermophilic bacterium *Thermotoga maritima* has two different classes of family C DNA polymerases: evolutionary implications

AUTHOR(S): Huang, Yi-Ping; Ito, Junetsu

CORPORATE SOURCE: Department of Microbiology and Immunology, The University of Arizona, Tucson, AZ, 85724, USA

SOURCE: Nucleic Acids Res. (1998), 26(23), 5300-5309

CODEN: NARHAD; ISSN: 0305-1048

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Bacterial DNA polymerase III (family C DNA polymerase), the principal chromosomal replicative enzyme, is known to occur in at least three distinct forms which have provisionally been classified as class I (*Escherichia coli* DNA pol C-type), class II (*Bacillus subtilis* DNA pol C-type) and class III (cyanobacteria DNA pol C-type). We have identified two family C ***DNA*** ***polymerase*** sequences in the hyperthermophilic bacterium ****Thermotoga**** ****maritima****. One DNA polymerase consisting of 842 amino acid residues and having a mol. wt. of 97 213 belongs to class I. The other one, consisting of 1367 amino acid residues and having a mol. wt. of 155 361, is a member of class II. Comparative sequence analyses suggest that the class II DNA polymerase is the principal DNA replicative enzyme of the microbe and that the class I DNA polymerase may be functionally inactive. A phylogenetic anal. using the class II enzyme indicates that *T. maritima* is closely related to the low G+C Gram-pos. bacteria, in particular to *Clostridium acetobutylicum*, and mycoplasmas. These results are in conflict with 16S rRNA-based phylogenies, which placed *T. maritima* as one of the deepest branches of the bacterial tree.

L3 ANSWER 11 OF 22 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 2

ACCESSION NUMBER: 1998:663452 CAPLUS

DOCUMENT NUMBER: 130:21096

TITLE: Capacity of nine thermostable DNA polymerases to
mediate DNA amplification in the presence of
PCR-inhibiting samples

AUTHOR(S): Al-Soud, Waleed Abu; Radstrom, Peter

CORPORATE SOURCE: Applied Microbiology, Center for Chemistry and
Chemical Engineering, Lund Institute of Technology,
Lund University, Lund, SE-221 00, Swed.

SOURCE: Appl. Environ. Microbiol. (1998), 64(10), 3748-3753

CODEN: AEMIDF; ISSN: 0099-2240

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The PCR is an extremely powerful method for detecting microorganisms.

However, its full potential as a rapid detection method is limited by the inhibition of the thermostable DNA polymerase from *Thermus aquaticus* by many components found in complex biol. samples. In this study, we have compared the effects of known PCR-inhibiting samples on nine thermostable DNA polymerases. Samples of blood, cheese, feces, and meat, as well as various ions, were added to PCR mixts. contg. various thermostable DNA polymerases. The nucleic acid amplification capacity of the nine polymerases, under buffer conditions recommended by the manufacturers, was evaluated by using a PCR-based detection method for *Listeria monocytogenes* in the presence of purified template DNA and different concns. of PCR inhibitors. The AmpliTaq Gold and the Taq DNA polymerases from *Thermus aquaticus* were totally inhibited in the presence of 0.004% (vol/vol) blood in the PCR mixt., while the HotTub, Pwo, rTth, and Tft DNA polymerases were able to amplify DNA in the presence of 20% (vol/vol) blood without reduced amplification sensitivity. The ***DNA*** ***polymerase*** from ****Thermotoga**** ****maritima**** (Ultma) was found to be the most susceptible to PCR inhibitors present in cheese, feces, and meat samples. When the inhibitory effect of K and Na ions was tested on the nine polymerases, HotTub from *Thermus flavus* and rTth from *Thermus thermophilus* were the most resistant. Thus, the PCR-inhibiting effect of various components in biol. samples can, to some extent, be eliminated by the use of the appropriate thermostable DNA polymerase.

L3 ANSWER 12 OF 22 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 3

ACCESSION NUMBER: 1998:747283 CAPLUS

DOCUMENT NUMBER: 130:120102

TITLE: Accuracy of replication in the polymerase chain
reaction. Comparison between ****Thermotoga****
****maritima**** ***DNA*** ***polymerase***
and *Thermus aquaticus* ***DNA*** ***polymerase***

AUTHOR(S): Diaz, R. S.; Sabino, E. C.

CORPORATE SOURCE: Laboratorio de Retrovirologia, Universidade Federal de
Sao Paulo, Sao Paulo, Brazil

SOURCE: Braz. J. Med. Biol. Res. (1998), 31(10), 1239-1242

CODEN: BJMRDK; ISSN: 0100-879X

PUBLISHER: Associacao Brasileira de Divulgacao Cientifica

DOCUMENT TYPE: Journal

LANGUAGE: English

AB For certain applications of the polymerase chain reaction (PCR), it may be

necessary to consider the accuracy of replication. The breakthrough that made PCR user friendly was the commercialization of *Thermus aquaticus* (Taq) DNA polymerase, an enzyme that would survive the high temps. needed for DNA denaturation. The development of enzymes with an inherent 3' to 5' exonuclease proofreading activity, lacking in Taq polymerase, would be an improvement when higher fidelity is needed. We used the forward mutation assay to compare the fidelity of Taq polymerase and ****Thermotoga**** ****maritima**** (ULTMATM) ***DNA*** ***polymerase***, an enzyme that does have proofreading activity. We did not find significant differences in the fidelity of either enzyme, even when using optimal buffer conditions, thermal cycling parameters, and no. of cycles (0.2% and 0.13% error rates for ULMATM and Taq, resp., after reading about 3,000 bases each). We conclude that for sequencing purposes there is no difference in using a DNA polymerase that contains an inherent 3' to 5' exonuclease activity for DNA amplification. Perhaps the specificity and fidelity of PCR are complex issues influenced by the nature of the target sequence, as well as by each PCR component.

L3 ANSWER 13 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1997:281157 CAPLUS

DOCUMENT NUMBER: 126:260879

TITLE: Amino acid substituted DNA polymerases from *Thermotoga* lacking exonuclease activities and their uses

INVENTOR(S): Chatterjee, Deb K.; Hughes, A. John, Jr.

PATENT ASSIGNEE(S): Chatterjee, Deb K., USA; Hughes, A. John, Jr.

SOURCE: PCT Int. Appl., 120 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 6

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9709451 | A1 | 19970313 | WO 1996-US14189 | 19960906 |
| W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM | | | | |
| US 5939301 | A | 19990817 | US 1995-537400 | 19951002 |
| AU 9672362 | A1 | 19970327 | AU 1996-72362 | 19960906 |
| EP 871775 | A1 | 19981021 | EP 1996-933753 | 19960906 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI | | | | |
| PRIORITY APPLN. INFO.: US 1995-525057 19950908 | | | | |
| US 1995-537397 19951002 | | | | |
| US 1995-537400 19951002 | | | | |
| US 1995-576759 19951221 | | | | |
| US 1996-689818 19960814 | | | | |
| US 1994-316423 19940930 | | | | |
| US 1995-370190 19950109 | | | | |
| WO 1996-US14189 19960906 | | | | |
| AB Analogs of thermostable Tne (*** <i>Thermotoga</i> *** *** <i>neapolitana</i> ***) | | | | |

and Tma (T. maritima) ***DNA*** ***polymerases*** of Thermotoga that have little or no 5'.fwdarw.3' or 3'.fwdarw.5' exonuclease activity or are less discriminating against 2',3'-dideoxynucleotides are described for use in DNA sequencing, labeling, amplification and cDNA synthesis. The Tne DNA polymerase has a mol. wt. of about 100,000 and is more thermostable than Taq DNA polymerase. The enzymes are manufd. by expression of the cloned gene in Escherichia coli. The genes for these enzymes were cloned by expression in E. coli. Amino acids essential for the exonuclease and discriminatory activities were identified by sequence comparison. Substitution and deletion analogs were prepd. by std. methods of mutagenesis and expression. Elimination of the exonuclease activities increased the readable length of DNA sequences to >400 bases and signal strength by about 5-fold.

L3 ANSWER 14 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1997:684232 CAPLUS

DOCUMENT NUMBER: 127:327443

TITLE: Nucleic acid amplification using a reversibly inactivated thermostable enzyme

INVENTOR(S): Birch, David Edward; Laird, Walter Joseph; Zoccoli, Michael Anthony

PATENT ASSIGNEE(S): Roche Molecular Systems, Inc., USA

SOURCE: U.S., 20 pp.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|----------|-----------------|----------|
| US 5677152 | A | 19971014 | US 1996-684108 | 19960719 |

OTHER SOURCE(S): MARPAT 127:327443

AB A thermostable enzyme is provided which is reversibly inactivated by chem. modification, characterized in that an incubation of the chem. modified thermostable enzyme in an aq. buffer at alk. pH at a temp. .ltorsim.25.degree. results in no significant increase in enzyme activity in .ltorsim.20 min, and wherein incubation of the chem. modified enzyme in an aq. buffer, formulated to about pH 8-9 at 25.degree., at a temp. .gtorsim.50.degree. results in a .gtoreq.2-fold increase in enzyme activity in .ltorsim.20 min. The chem. modified thermostable enzyme can be used for the amplification of nucleic acids, whereby the activity of the inactivated enzyme is recovered by an incubation of the reaction mixt. at an elevated temp. prior to, or as part of, the amplification reaction. The modification reagent is a dicarboxylic acid anhydride such as maleic anhydride, citraconic anhydride, cis-aconitic anhydride, 2,3-dimethylmaleic anhydride, 3,4,5,6-tetrahydrophthalic anhydride, or exo-cis-3,6-endoxo- DELTA.4-tetrahydrophthalic anhydride.

L3 ANSWER 15 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1997:347209 CAPLUS

DOCUMENT NUMBER: 126:313176

TITLE: Nucleic acid amplification using a reversibly inactivated thermostable enzyme

INVENTOR(S): Birch, David Edward; Laird, Walter Joseph; Zoccoli, Michael Anthony

PATENT ASSIGNEE(S): F.Hoffmann-La Roche Ag, Switz.
SOURCE: Can. Pat. Appl., 37 pp.
CODEN: CPXXEB
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-------------------|----------|
| CA 2184105 | AA | 19970226 | CA 1996-2184105 | 19960823 |
| EP 771870 | A1 | 19970507 | EP 1996-113222 | 19960817 |
| EP 771870 | B1 | 19990203 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, SE | | | | |
| AT 176499 | E | 19990215 | AT 1996-113222 | 19960817 |
| ES 2101668 | T3 | 19990701 | ES 1996-113222 | 19960817 |
| AU 9662179 | A1 | 19970313 | AU 1996-62179 | 19960821 |
| AU 689047 | B2 | 19980319 | | |
| NO 9603541 | A | 19970226 | NO 1996-3541 | 19960823 |
| CN 1151437 | A | 19970611 | CN 1996-113219 | 19960825 |
| JP 09103292 | A2 | 19970422 | JP 1996-240996 | 19960826 |
| BR 9603563 | A | 19980519 | BR 1996-3563 | 19960826 |
| PRIORITY APPLN. INFO.: | | | US 1995-2673 | 19950825 |
| OTHER SOURCE(S): | | | MARPAT 126:313176 | |

AB A thermostable enzyme is provided which is reversibly inactivated by chem. modification, characterized in that an incubation of the chem. modified thermostable enzyme in an aq. buffer at alk. pH at a temp. .ltorsim.25.degree. results in no significant increase in enzyme activity in .ltorsim.20 min, and wherein incubation of the chem. modified enzyme in an aq. buffer, formulated to about pH 8-9 at 25.degree., at a temp. .gtorsim.50.degree. results in a .gtoreq.2-fold increase in enzyme activity in .ltorsim.20 min. The chem. modified thermostable enzyme can be used for the amplification of nucleic acids, whereby the activity of the inactivated enzyme is recovered by an incubation of the reaction mixt. at an elevated temp. prior to, or as part of, the amplification reaction. The modification reagent is a dicarboxylic acid anhydride such as maleic anhydride, citraconic anhydride, cis-aconitic anhydride, 2,3-dimethylmaleic anhydride, 3,4,5,6-tetrahydrophthalic anhydride, or exo-cis-3,6-endoxo-DELTA.4-tetrahydrophthalic anhydride. For example, citraconylation of Taq DNA polymerase yields a modified enzyme with the desired properties for nucleic acid amplification in a PCR system.

L3 ANSWER 16 OF 22 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:168532 CAPLUS
DOCUMENT NUMBER: 126:154434
TITLE: Thermophilic ***DNA*** ***polymerases*** from
Thermotoga ***neapolitana***
INVENTOR(S): Slater, Michael R.; Huang, Fen; Hartnett, James R.;
Bolchakova, Elena; Storts, Douglas R.; Otto, Paul;
Miller, Katharine M.
PATENT ASSIGNEE(S): Promega Corporation, USA
SOURCE: PCT Int. Appl., 200 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9641014 | A1 | 19961219 | WO 1996-US9641 | 19960607 |
| W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG | | | | |
| RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN | | | | |
| US 6001645 | A | 19991214 | US 1995-484661 | 19950607 |
| AU 9662640 | A1 | 19961230 | AU 1996-62640 | 19960607 |
| AU 705179 | B2 | 19990520 | | |
| EP 873420 | A1 | 19981028 | EP 1996-921407 | 19960607 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI | | | | |
| PRIORITY APPLN. INFO.: | | | US 1995-484661 | 19950607 |
| | | | US 1996-656664 | 19960531 |
| | | | WO 1996-US9641 | 19960607 |

AB Thermostable DNA polymerases are provided derived from the hyperthermophilic eubacterium known as *Thermotoga neapolitana* (Tne). The wild-type gene was isolated and sequenced and shown to code for a 893-amino-acid enzyme. Specific alterations of the Tne polymerase gene were: deletions between residues 1-849, 1-945, 1-966, and 1-849 and 924-1272; and substitutions at residues 945, 947, 967, 968, 975, 1166, 1167, 1391, 1402, 1407, 1410, 2184, 2189. To construct mutant Tne polymerases lacking 5' to 3' exonuclease activity, deletions mutants of the Tne polymerase gene were generated which removed sequences encoding a large portion of the 5' to 3' exonuclease domain located at the N-terminus of the Tne polymerase. Modified forms of the Tne polymerase which possess varying amts. of 3' to 5' exonuclease activity, 7 different point mutants and 2 deletions mutants were created. The Tne Quad polymerase comprises the deletion of residues 1-283 from the N-terminus and contains e amino substitutions at residues 323 (alanine), 389 (alanine), and 730 (tyrosine). The modified Tne polymerases utilize a broader range of optimal deoxynucleotide triphosphate concns. in PCR, tolerate a broader range of Mg²⁺, and have improved characteristics for applications such as thermal cycle sequencing, PCR, and long PCR. Redn. in 3' exonuclease results in a lowered fidelity for the modified Tne polymerases, which is advantageous when mutagenic PCR is to be performs. Addn. of a small amt. of the high fidelity *Thermococcus litoralis* DNA polymerase to the modified Tne polymerases greatly improves the fidelity of the overall reaction. The T. neapolitana DNA polymerases of the present invention are used in combination with other compds., including but not limited to pyrophosphatase and DNA polymerases from other thermophilic or hyperthermophilic organisms.

L3 ANSWER 17 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1996:388345 CAPLUS

DOCUMENT NUMBER: 125:52372

TITLE: Cloning of gene for thermostable ***DNA***
 polymerases from ***Thermotoga***
 neapolitana and mutants thereof
 characterization of the enzymes

INVENTOR(S): Hughes, A. John, Jr.; Chatterjee, Deb K.

PATENT ASSIGNEE(S): Life Technologies, Inc., USA
SOURCE: PCT Int. Appl., 66 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 6
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9610640 | A1 | 19960411 | WO 1995-US12358 | 19951002 |
| W: CA, JP | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE | | | | |
| US 5912155 | A | 19990615 | US 1995-370190 | 19950109 |
| EP 725827 | A1 | 19960814 | EP 1995-935150 | 19951002 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE | | | | |
| JP 09506783 | T2 | 19970708 | JP 1995-511997 | 19951002 |
| PRIORITY APPLN. INFO.: US 1994-316423 19940930 | | | | |
| US 1995-370190 19950109 | | | | |
| WO 1995-US12358 19951002 | | | | |

AB The invention relates to a substantially pure thermostable ***DNA***
polymerase from ***Thermotoga*** ***neapolitana*** (Tne)
and mutants thereof. The Tne DNA polymerase has a mol. wt. of about 100
kilodaltons and is more thermostable than Taq DNA polymerase. The mutant
Tne DNA polymerase has at least one mutation selected from the group
consisting of (1) a first mutation that substantially reduces or
eliminates 3' .fwdarw. 5' exonuclease activity of said DNA polymerase; (2)
a second mutation that substantially reduces or eliminates 5' .fwdarw. 3'
exonuclease activity of said DNA polymerase; (3) a third mutation in the O
helix of said DNA polymerase resulting in said DNA polymerase becoming
non-discriminating against dideoxynucleotides. The present invention also
relates to the cloning and expression of the wild type or mutant Tne DNA
polymerase in E. coli, to DNA mols. contg. the cloned gene, and to host
cells which express said genes. The Tne DNA polymerase of the invention
may be used in well-known DNA sequencing and amplification reactions.

L3 ANSWER 18 OF 22 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1996:590888 CAPLUS
DOCUMENT NUMBER: 125:214263
TITLE: Combination of exonuclease-positive DNA polymerase and
exonuclease-negative DNA polymerase in improved
polymerase chain reaction
INVENTOR(S): Sorge, Joseph A.; Mullinax, Rebecca L.
PATENT ASSIGNEE(S): Stratagene, USA
SOURCE: U.S., 26 pp. Cont.-in-part of U.S. Ser. No. 164, 290.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|----------|-----------------|----------|
| US 5556772 | A | 19960917 | US 1994-197791 | 19940216 |
| WO 9516028 | A1 | 19950615 | WO 1994-US14065 | 19941207 |
| W: CA, JP | | | | |

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRIORITY APPLN. INFO.: US 1993-164290 19931208
US 1994-197791 19940216

AB The subject invention provides novel compns. contg. a mixt. of (a) an enzyme that possesses substantial 3'-5' exonuclease activity (b) a DNA polymerase with less 3'-5' exonuclease activity than the enzyme with substantial 3'-5' exonuclease activity. Preferably, the DNA polymerase for inclusion in the compns. are DNA polymerases that substantially lack 3'-5' exonuclease activity. A preferred embodiment of the invention is a compn. comprising the Taq DNA polymerase (isolated from *Thermus aquaticus*) and the Pfu DNA polymerase (isolated from *Pyrococcus furiosus*). Another aspect of the invention is to provide methods for synthesizing polynucleotides, typically DNA, using compns. comprising an enzyme that possesses substantial 3'-5' exonuclease activity and a DNA polymerase with less 3'-5' exonuclease activity than the enzymes possessing substantial 3'-5' exonuclease activity, preferably a DNA polymerase that substantially lacks 3'-5' exonuclease activity. Another aspect of the invention involves the use the subject method of polynucleotide synthesis to carry out the synthesis step in a polymerase chain reaction expt. Yet another aspect of the invention is to provide kits for the synthesis of polynucleotides, wherein the kits comprise an enzyme that possesses substantial 3'-5' exonuclease activity and a DNA polymerase with less 3'-5' exonuclease activity than the enzyme possessing substantial 3'-5' exonuclease activity. Expts. using Taq (*Thermus aquaticus*; 3'.fwdarw.5' exonuclease-neg.) and Pfu (*Pyrococcus furiosus*; 3'.fwdarw.5' exonuclease-pos.) DNA polymerases were conducted. Using hybridoma and peripheral blood lymphocyte templates and primers contg. 0, 1 or 2 3'-mismatches, Taq polymerase could only extend those primers not contg. mismatches under the conditions used. The combination of Taq and Pfu polymerases allowed extension of all primers and resulted in more product in some samples. The effects of Taq to Pfu polymerase ratios, template concn., and annealing temp. were also examd.

L3 ANSWER 19 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1995:377249 CAPLUS

DOCUMENT NUMBER: 122:153369

TITLE: Truncated *Thermus* DNA polymerases with enhanced
thermostability and DNA polymerase formulations for
enhancement of nucleic acid amplification

INVENTOR(S): Barnes, Wayne M.

PATENT ASSIGNEE(S): USA

SOURCE: PCT Int. Appl., 78 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|------|-----------------|------|
|------------|------|------|-----------------|------|

| | | | | |
|------------|----|----------|----------------|----------|
| WO 9426766 | A1 | 19941124 | WO 1994-US1867 | 19940222 |
|------------|----|----------|----------------|----------|

W: AU, CA, JP, NZ

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

| | | | | |
|------------|---|----------|---------------|----------|
| US 5436149 | A | 19950725 | US 1993-21623 | 19930219 |
|------------|---|----------|---------------|----------|

| | | | | |
|------------|----|----------|-----------------|----------|
| CA 2156176 | AA | 19941124 | CA 1994-2156176 | 19940222 |
|------------|----|----------|-----------------|----------|

| | | | | |
|------------|----|----------|---------------|----------|
| AU 9462464 | A1 | 19941212 | AU 1994-62464 | 19940222 |
|------------|----|----------|---------------|----------|

AU 671204 B2 19960815
 EP 693078 A1 19960124 EP 1994-909742 19940222
 EP 693078 B1 19990623
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE
 JP 11501801 T2 19990216 JP 1994-522506 19940222
 JP 2885324 B2 19990419
 AT 181573 E 19990715 AT 1994-909742 19940222
 JP 11239492 A2 19990907 JP 1998-359199 19940222
 PRIORITY APPLN. INFO.: US 1993-21623 19930219
 US 1994-202032 19940222
 JP 1994-522506 19940222
 WO 1994-US1867 19940222

AB A DNA polymerase having an amino acid sequence comprising substantially the same amino acid sequence as that of *Thermus aquaticus* or *Thermus flavus* DNA polymerase, excluding the N-terminal 280 amino acid residues of *Thermus aquaticus* DNA polymerase or the N-terminal 279 amino acid residues of *Thermus flavus* DNA polymerase, and recombinant DNA sequences encoding said DNA polymerases are claimed. A formulation of thermostable or other DNA polymerases comprising a majority component comprised of at least one thermostable or other DNA polymerase of the type described above, wherein the DNA polymerase lacks 3'-exonuclease activity, and a minority component comprised of at least one thermostable DNA polymerase exhibiting 3'-exonuclease activity, and an improved method for enzymic extension of DNA strands, esp. while, but not limited to, amplifying nucleic acid sequences by polymerase chain reaction wherein the above formulation is made and used to catalyze primer extension, are also provided. Expression vector pWB254, encoding KlenTaq-278 (the *T. aquaticus* DNA polymerase deriv.), was prepd. *E. coli* contg. this plasmid were used to prep. the enzyme and large-scale purifn. of the enzyme was performed. In a PCR expt., exposure to 98.degree. was not detectably detrimental to KlenTaq-278. Using a 640:1 mixt. of this enzyme with *Pyrococcus furiosus* DNA polymerase, efficient amplification of 8.4, 12.5, 15, and 18 kb DNA fragments was demonstrated. The fidelity of the product amplified was at least equal to that obtained using *P. furiosus* DNA polymerase alone.

L3 ANSWER 20 OF 22 CAPLUS-COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1995:367685 CAPLUS

DOCUMENT NUMBER: 122:153388

TITLE: Cloning and expression of ****Thermotoga****
 ****maritima**** gene for thermostable ***DNA***
 polymerase

INVENTOR(S): Gelfand, David H.; Lawyer, Frances C.

PATENT ASSIGNEE(S): Hoffmann-la Roche Inc., USA

SOURCE: U.S., 22 pp. Cont.-in-part of U.S. Ser. No.143,441,
 abandoned.

CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 26

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|------------|------|----------|-----------------|----------|
| US 5374553 | A | 19941220 | US 1990-567244 | 19900813 |
| US 4889818 | A | 19891226 | US 1987-63509 | 19870617 |
| CA 2089495 | AA | 19920214 | CA 1991-2089495 | 19910813 |

WO 9203556 A1 19920305 WO 1991-US5753 19910813
W: AU, CA, JP, US
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE
AU 9185014 A1 19920317 AU 1991-85014 19910813
AU 653747 B2 19941013
EP 544789 A1 19930609 EP 1991-915802 19910813
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE
JP 06500020 T2 19940106 JP 1991-514681 19910813
JP 07108220 B4 19951122
US 5352600 A 19941004 US 1992-971798 19921105
US 5420029 A 19950530 US 1993-971819 19930203
US 5407800 A 19950418 US 1993-80243 19930617
US 5455170 A 19951003 US 1993-113531 19930827
US 5618703 A 19970408 US 1994-199509 19940222
US 5641864 A 19970624 US 1994-311612 19940922
JP 07147990 A2 19950613 JP 1994-253968 19941019
JP 2584198 B2 19970219
US 5618711 A 19970408 US 1995-384490 19950206
US 5789224 A 19980804 US 1995-459383 19950602
US 5795762 A 19980818 US 1995-458819 19950602
US 5674738 A 19971007 US 1995-520422 19950829
JP 08298991 A2 19961119 JP 1996-117847 19960513
US 1986-899241 19860822
PRIORITY APPLN. INFO.:

US 1987-63509 19870617
US 1988-143441 19880112
US 1989-387174 19890728
US 1989-455611 19891222
US 1989-455967 19891222
US 1990-523394 19900515
US 1990-557517 19900724
US 1990-567244 19900813
US 1990-585471 19900920
US 1990-590213 19900928
US 1990-590466 19900928
US 1990-590490 19900928
US 1990-609157 19901102
JP 1994-253968 19910813
WO 1991-US5753 19910813
US 1991-746121 19910815
US 1992-880478 19920506
US 1993-977434 19930223
US 1993-82182 19930624
US 1993-113531 19930827
US 1993-148133 19931102
US 1994-199509 19940222
US 1995-384490 19950206

AB DNA encoding the title enzyme, expression vectors contg. the DNA, host cells contg. the expression vectors, and manuf. of the DNA polymerase with these recombinant cells are claimed. The enzyme has a mol. wt. of about 97 kD and DNA polymerase I activity. The purified enzyme was used in a PCR. Expression vectors for Escherichia coli were prepd.

L3 ANSWER 21 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1993:118290 CAPLUS

DOCUMENT NUMBER: 118:118290

TITLE: Analogs of a thermostable DNA polymerases with altered

5'.fwdarw.3' exonuclease activity and their
manufacture

INVENTOR(S): Gelfand, David H.; Abramson, Richard D.

PATENT ASSIGNEE(S): Cetus Corp., USA

SOURCE: PCT Int. Appl., 85 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 26

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|------|----------|-----------------|----------|
| WO 9206200 | A1 | 19920416 | WO 1991-US7035 | 19910930 |
| W: AU, CA, JP, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE | | | | |
| CA 2090614 | AA | 19920329 | CA 1991-2090614 | 19910930 |
| AU 9186688 | A1 | 19920428 | AU 1991-86688 | 19910930 |
| AU 663474 | B2 | 19951012 | | |
| EP 550687 | A1 | 19930714 | EP 1991-919358 | 19910930 |
| EP 550687 | B1 | 19990609 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| JP 05506364 | T2 | 19930922 | JP 1991-516787 | 19910930 |
| JP 10000095 | A2 | 19980106 | JP 1997-70200 | 19910930 |
| JP 10004985 | A2 | 19980113 | JP 1997-70163 | 19910930 |
| JP 10004965 | A2 | 19980113 | JP 1997-70192 | 19910930 |
| EP 894860 | A1 | 19990203 | EP 1998-115951 | 19910930 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| AT 181106 | E | 19990615 | AT 1991-919358 | 19910930 |
| ES 2134198 | T3 | 19991001 | ES 1991-919358 | 19910930 |
| US 5466591 | A | 19951114 | US 1993-977434 | 19930223 |
| US 5455170 | A | 19951003 | US 1993-113531 | 19930827 |
| US 5795762 | A | 19980818 | US 1995-458819 | 19950602 |
| US 5674738 | A | 19971007 | US 1995-520422 | 19950829 |
| AU 9640868 | A1 | 19960426 | AU 1996-40868 | 19960108 |
| AU 691374 | B2 | 19980514 | | |

PRIORITY APPLN. INFO.: US 1990-590213 19900928

US 1990-590466 19900928
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US 1986-899241 19860822
US 1987-63509 19870617
US 1988-143441 19880112
US 1989-455611 19891222
US 1990-523394 19900515
US 1990-557517 19900724
US 1990-585471 19900920
US 1990-609157 19901102
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EP 1991-919358 19910930
JP 1991-516787 19910930
WO 1991-US7035 19910930
US 1993-977434 19930223
US 1993-113531 19930827

AB Thermostable DNA polymerase mutants with greater or lesser 5'.fwdarw.3' exonuclease activity are prep'd. by expression of the corresponding genes in Escherichia coli. The genes for the thermostable DNA polymerases are

selected from *Thermus* sps17, *Thermus* Z05, *Thermus aquaticus*, *Thermus thermophilus*, *Thermosiphon africanus*, and *Thermotoga maritima* and are mutagenized by substitution or deletion involving site-specific mutation and polymerase chain reaction (PCR). Prepn. of analogs of the Taq DNA polymerase of *Thermus aquaticus* and other species was demonstrated and their defined nucleotide and amino acid sequences given.

L3 ANSWER 22 OF 22 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1992:546202 CAPLUS

DOCUMENT NUMBER: 117:146202

TITLE: A thermostable ***DNA*** ***polymerase*** I
from ****Thermotoga**** ****maritima****

INVENTOR(S): Gelfand, David H.; Lawyer, Frances C.; Stoffel,
Susanne

PATENT ASSIGNEE(S): Cetus Corp., USA

SOURCE: PCT Int. Appl., 67 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

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| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
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| WO 9203556 | A1 | 19920305 | WO 1991-US5753 | 19910813 |
| W: AU, CA, JP, US | | | | |
| RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, NL, SE | | | | |
| US 5374553 | A | 19941220 | US 1990-567244 | 19900813 |
| AU 9185014 | A1 | 19920317 | AU 1991-85014 | 19910813 |
| AU 653747 | B2 | 19941013 | | |
| EP 544789 | A1 | 19930609 | EP 1991-915802 | 19910813 |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE | | | | |
| JP 06500020 | T2 | 19940106 | JP 1991-514681 | 19910813 |
| JP 07108220 | B4 | 19951122 | | |
| US 5420029 | A | 19950530 | US 1993-971819 | 19930203 |
| PRIORITY APPLN. INFO.: | | | | US 1990-567244 19900813 |
| | | | | US 1986-899241 19860822 |
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| | | | | US 1988-143441 19880112 |
| | | | | WO 1991-US5753 19910813 |


AB A thermostable DNA polymerase I is obtained from the anaerobic hyperthermophilic bacterium *Thermotoga maritima* and the corresponding gene cloned and expressed in *Escherichia coli*. The enzyme is useful in polymerase chain reaction (PCR) and other temp.-cycling amplification nucleic acid amplification methods. The enzyme was purified from cell lysates chromatog. The gene was then cloned by PCR using primers derived from conserved sequences of thermostable DNA polymerases to obtain a fragment that was used as a probe to screen a gene bank. The gene was expressed in *E. coli* from the .lambda. PL promoter. The protein, or an N-terminal deletion analog of it lacking the N-terminal 283 amino acids had half-lives at 95.degree. >2-fold longer than that of Taq polymerase.

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| | U | Document ID | Issue Date | Pages | Title | Current OR |
|----|-------------------------------------|--|------------|-------|---|------------|
| 1 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 6008025 A | 19991228 | 33 | Modified thermostable DNA polymerase derived from <i>pyrococcus</i> sp. KOD and DNA polymerase composition thereof for nucleic acid amplification | 435/91.2 |
| 2 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5994056 A | 19991130 | 23 | Homogeneous methods for nucleic acid amplification and detection | 435/6 |
| 3 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5968799 A | 19991019 | 30 | Purified thermostable nucleic acid polymerase enzyme from <i>thermosiph</i> | 435/194 |
| 4 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5795762 A | 19980818 | 65 | aficantus 5' to 3' exonuclease mutations of thermostable DNA polymerases | 435/194 |
| 5 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5773258 A | 19980630 | 20 | Nucleic acid amplification using a reversibly inactivated thermostable enzyme | 435/91.2 |
| 6 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5766888 A | 19980616 | 25 | Detection of carcinoma metastases by nucleic acid amplification | 435/91.2 |
| 7 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5693517 A | 19971202 | 36 | Reagents and methods for coupled high temperature reverse transcription | 435/193 |
| 8 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5677152 A | 19971014 | 20 | and polymerase chain reactions Nucleic acid amplification using a reversibly inactivated thermostable enzyme | 435/91.2 |
| 9 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5674738 A | 19971007 | 27 | DNA encoding thermostable nucleic acid polymerase enzyme from <i>thermus</i> | 435/252.3 |
| 10 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5641864 A | 19970624 | 29 | species Z05 Kits for high temperature reverse transcription of RNA | 530/350 |

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| 11 | <input type="checkbox"/> | <input checked="" type="checkbox"/> US 5624833 A | 19970429 | 38 | Purified thermostable nucleic acid polymerase enzyme from <i>Thermotoga</i> | 435/194 |
| 12 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5620847 A | 19970415 | 47 | Therminia Methods and reagents for detection of bacteria in cerebrospinal fluid | 435/6 |
| 13 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5618703 A | 19970408 | 28 | Unconventional nucleotide substitution in temperature selective RT-PCR | 435/91.2 |
| 14 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5602011 A | 19970211 | 19 | Purified <i>Thermococcus barosii</i> DNA polymerase | 435/91.2 |
| 15 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5561058 A | 19961001 | 41 | Methods for coupled high temperatures reverse transcription and | 435/91.2 |
| 16 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5556772 A | 19960917 | 26 | polymerase chain reactions Polymerase compositions and uses thereof | 435/91.2 |
| 17 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5543296 A | 19960806 | 18 | Detection of carcinoma metastases by nucleic acid amplification | 435/6 |
| 18 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5512462 A | 19960430 | 19 | Methods and reagents for the polymerase chain reaction amplification of | 435/91.2 |
| 19 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5466591 A | 19951114 | 67 | long-DNA sequences 5' to 3' exonuclease mutations of thermostable DNA polymerases | 435/194 |
| 20 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5455170 A | 19951003 | 23 | Mutated thermostable nucleic acid polymerase enzyme from <i>Thermus</i> species | 435/252.3 |
| 21 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> US 5420029 A | 19950530 | 40 | Z05 Mutated thermostable nucleic acid polymerase enzyme from <i>thermotoga</i> | 435/194 |

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|----|-------------------------------------|---|--------------|------------|-------|---|------------|
| 22 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | US 5407800 A | 19950418 | 24 | Reverse transcription with <i>Thermus thermophilus</i> polymerase | 435/6 |
| 23 | <input type="checkbox"/> | <input checked="" type="checkbox"/> | US 5374553 A | 19941220 | 22 | DNA encoding a thermostable nucleic acid polymerase enzyme from | 435/252.3 |
| 24 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | US 5314809 A | 19940524 | 16 | thermotoga maritima Methods for nucleic acid amplification | 435/91.2 |
| 25 | <input checked="" type="checkbox"/> | <input checked="" type="checkbox"/> | US 5310652 A | 19940510 | 26 | Reverse transcription with thermostable DNA polymerase-high temperature | 435/6 |
| 26 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 6015668 A | 20000118 | 65 | reverse transcription Cloned DNA polymerases from <i>thermotoga</i> and mutants thereof | 435/6 |
| 27 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 6001645 A | 19991214 | | Thermophilic DNA polymerases from <i>thermotoga neapolitana</i> | 435/320.1 |
| 28 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 5948614 A | 19990907 | 67 | Cloned DNA polymerases from <i>thermotoga maritima</i> and mutants thereof | 435/6 |
| 29 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 5939292 A | 19990817 | | Thermostable DNA polymerases having reduced discrimination against ribo-NTPs | 435/91.2 |

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| 1 | <input type="checkbox"/> | <input type="checkbox"/> | US 6015668 A | 20000118 | 65 | Cloned DNA polymerases from thermotoga and mutants thereof | 435/6 |
| 2 | <input type="checkbox"/> | <input type="checkbox"/> | US 6001645 A | 19991214 | 90 | Thermophilic DNA polymerases from thermotoga neapolitana | 435/320.1 |
| 3 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 5948614 A | 19990907 | 67 | Cloned DNA polymerases from thermotoga maritima and mutants thereof | 435/6 |
| 4 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 5939301 A | 19990817 | 35 | Cloned DNA polymerases from Thermotoga neapolitana and mutants thereof | 435/194 |
| 5 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 5939292 A | 19990817 | 19 | Thermostable DNA polymerases having reduced discrimination against | 435/91.2 |
| 6 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 5912155 A | 19990615 | 17 | Cloned DNA polymerases from Thermotoga neapolitana | 435/194 |
| 7 | <input checked="" type="checkbox"/> | <input type="checkbox"/> | US 5861295 A | 19990119 | 12 | Nucleic acid-free thermostable enzymes and methods of production thereof | 435/194 |

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